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Brief Overview of Selected Aspects of Testicular Hormone Action

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This paper is designed to give an overview of the mechanism of androgen action and some of the factors that can affect it. The discussion of androgen action includes androgen transport in the blood, metabolism, receptor binding, nuclear activation and selected aspects of biological response. The importance of recognizing interspecies and interstrain differences in specific aspects of androgen action is mentioned. Some examples of the effects of environmental agents on androgen metabolism, receptor binding and biological response are included.

Introduction

It has been recognized for some time that toxins can have far-reaching effects within the human body. Although it is only now coming under study, hormone action may be particularly susceptible to environmental factors due to the many steps involved in its manifestation. The testis is known to secrete several different hormones. The actions of two of them, inhibin and mullerian inhibiting factor, are not well defined and I will not discuss them further. The action of estrogen, which may be a major testicular product in some species, will be discussed in a later session.

The major focus of this paper will be on the mechanism of androgen action. Space does not permit me to go into the intricate details involved. These can be obtained from numerous reviews. Instead, I want to give an overview and to elucidate some general points that should be considered by toxicologists studying the mechanism of, or the effects of any compound on, androgen action.

It is necessary to consider a number of different aspects when evaluating a compound for its effect on androgen action. The biological efficacy of an androgen is influenced by its rate of synthesis or entry into the circulation, its metabolism in target and peripheral tissues, its availability for uptake by the target organs as well as its biochemical action within the target cell itself.

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In my study of androgen action, I have used primarily the mouse kidney (*1-5*). This organ offers several advantages. Although it is not androgen-dependent for its differentiation, it is androgen-responsive. Androgen receptors are present in both males and females. The kidney responds to androgens with cellular hypertrophy, but not hyperplasia, of Bowman's capsule and the proximal convoluted tubule. The absence of cell division means a constant population of cells is available for study. The activity of a number of specific enzymes including β-glucuronidase, β-galactosidase, d-amino acid oxidase, alcohol dehydrogenase, and arginase are increased by androgens. There are a number of genetic mutants, with altered androgen responsiveness, which provide powerful tools for dissecting the regulation of androgen action (*6-8*). We have made extensive use of the androgen-insensitive Tfm/Y mouse (*3*). The androgen insensitivity is a result of the effective absence of androgen receptors. Although male accessory sex tissues do not develop, several non-androgen-dependent tissues remain which are androgen-responsive in the normal animal. The Tfm/Y mouse has been extremely useful for determining those effects that are mediated via the androgen receptor.

Circulation

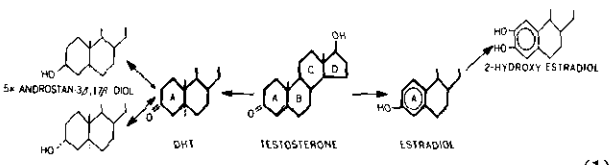
Some of the factors affecting testicular androgen biosynthesis were discussed by Ewing (*9*). Once secreted into the blood, free androgens circulate in

equilibrium with androgens bound to plasma binding proteins. The plasma protein with the highest binding affinity for androgens is sex hormone binding globulin (TeBG or SHBG). This protein is present in some species such as man, nonhuman primates, rabbits, and sheep but not in others including rodents, dogs, cats, and birds (*10*). In these latter species, testosterone is bound primarily to albumin. Some potent synthetic androgens, such as R1881, are not bound by TeBG which may, in part, account for their increased androgenicity. Any agents, such as estrogens or drugs that alter the synthesis and thus, the plasma concentration, of hormone binding proteins will ultimately affect the rates of testosterone biosynthesis and metabolism (*11*, *12*).

Metabolism

Androgens are metabolized in the liver, lung and other peripheral tissues as well as in the target organs themselves. In the liver and lung, this metabolism is primarily degradative. Changes in degradative enzymatic activities can alter the metabolic clearance rate and, thus, the effective biological activity of a hormone. While some hepatic steroid metabolizing enzymes function constitutively, the activity of others may be affected by hormones, drugs or toxins (*13-15*). Some hepatic enzymes need a defined hormonal milieu during a critical neonatal period for normal activity to develop in the adult (*15*). Species differences in the mode of regulation and activity of hepatic steroid metabolizing enzymes may result in differences between species in the biological activity of androgens. We have reported marked species differences in the clearance rate and the effect on hepatic metabolism of several synthetic progestins known to affect androgen action (*16*). These differences in hepatic metabolism are just one reason why data on biological activity gained in one species, or for that matter one strain, does not necessarily apply to another.

In target organs testosterone may be reduced to 5α-dihydrotestosterone or aromatized to estrogens [Eq. (1)]. In initial studies of nuclear androgen



(1)

uptake in rat prostate ^{3}H-5α-dihydrotestosterone (DHT) was found after an injection of ^{3}H-testosterone (*17*). Thus, DHT was thought to be the active endogenous male hormone. This is in contrast to estrogen and progesterone target tissues, in which the active steroid is uniformly the major steroid secreted by the gonad. However, when we injected ^{3}H-testosterone into mice we found only unmetabolized ^{3}H-testosterone in renal nuclei (Fig. 1) (*5*). Subsequent work by ourselves and others have demonstrated that in androgen target tissues, the active steroid may be testosterone or DHT depending on the presence or absence of the enzyme 5α-reductase (*18*). As data have accumulated it has become evident that not only are there differences in the active androgen between tissues of the same species but that there may also be differences between species when only a single tissue is compared. Generally, in the adult animal, 5α-reductase is low to absent in kidney, muscle, submandibular gland and testis and present in skin, accessory sex tissues and portions of the brain and pituitary. In those tissues that lack 5α-reductase, exogenous administration of DHT will also induce nuclear uptake and androgenic responses in these tissues (Fig. 1), since the androgen receptor will bind either testosterone or DHT with high affinity. Since the biological responses to all steroids are initiated in the nucleus, it is important to determine the active intranuclear form of any compound that works via a steroid receptor. The compound injected or found in highest concentration in the blood is not necessarily what is concentrated in the nucleus.

An interesting example of the importance of target organ metabolism in determining the active steroid is given by the study of Wilson and colleagues on the sexual differentiation of the human and rabbit fetus (*19*, *20*). They showed that, although DHT is the active androgen in all male accessory sex tissues in the adult, *in utero*, testos-

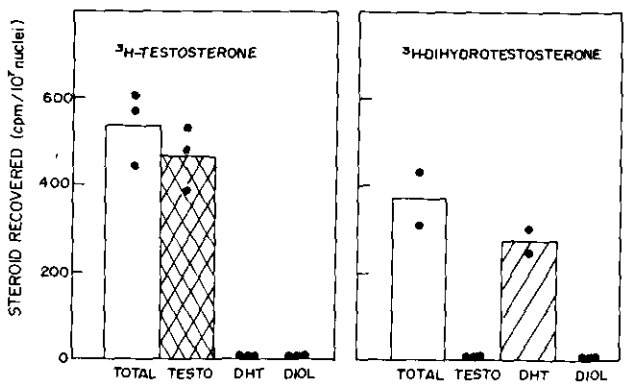


FIGURE 1. ^{3}H-androgens isolated from female mouse kidney nuclei following the IV administration of the ^{3}H-steroid indicated (*5*).

terone must be the active androgen for inducing the differentiation of Wolffian ducts. This is due to the fact that although 5α-reductase activity is present in the urogenital sinus and external genitalia at the time of differentiation, the enzyme does not become active until later in Wolffian ducts. The requirement for 5α-DHT for the differentiation of some accessory sex tissues is demonstrated by men with an inherited defect in 5α-reductase activity (*21*). Affected patients have testes as well as epididymidies, vas and seminal vesicles derived from Wolffian ducts. However, due to the absence of DHT, they lack well differentiated prostate and male external genitalia.

DHT may be further metabolized to the weakly androgenic 3α- and 3β-androstanediols. These compounds may derive their activity through back-conversion to DHT, as in the mouse kidney, or they may have direct effects as has been reported for the prostate (*18*). It is important to be aware of the activity of steroid-metabolizing enzymes in target tissues in any study of steroid action in which steady state kinetics is required. We found that, although testosterone was not metabolized in mouse kidney, DHT was converted to the androstanediols at a rapid rate even at 4°C (Fig. 2) (*1*).

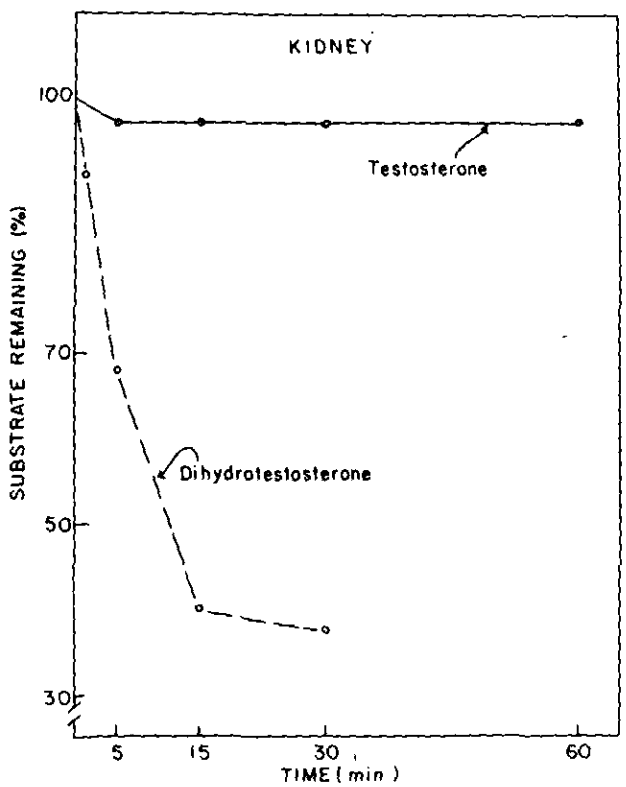


FIGURE 2. Mouse kidney slices were incubated (37°C) with either ^{3}H-testosterone or ^{3}H-dihydrotestosterone and the substrate remaining quantified (*4*).

The importance of aromatization of testosterone in the sexual differentiation and subsequent function of the brain was first suggested by Naftolin et al. in 1972 and since then has received a great deal of attention (*22, 23*). Information on the role of sex steroids in the sexual development of the brain has been difficult to obtain, however. Use of human and rodent genetic mutants, enzyme blockers, and routine metabolic studies, indicate that although there are species differences, testosterone, DHT, and the estrogens have specific, essential roles in the normal development of the sexual function of the brain, and thus, secondarily, the reproductive tract as well as other peripheral tissues of both males and females. Aromatase activity, converting testosterone to estradiol, is highest in those parts of the brain that have been associated with regulation of reproductive function, i.e., the anterior hypothalamus, and preoptic area and a portion of the amygdala. Critical periods exist during fetal and neonatal life during which the proper hormone must be present if normal sexual differentiation of the brain is to occur. The results of this early differentiation may not be manifested until later when the animal goes through puberty.

The recognition that the brain can further metabolize estrogens to catechol estrogens extends the potential sphere of influence that testosterone may have within this organ. Catechol estrogens may act as competitive inhibitors of the inactivation of catecholamines by acting as substrate for catechol-O-methyltransferase. They, thus, have the potential to influence the biosynthesis and inactivation of dopamine and norepinephrine. Catechol estrogens have also been reported to affect gonadotropin regulation.

From the above discussion it should be apparent that metabolism of a steroid plays an important part in determining its activity. Any factor which alters this metabolism, whether it be peripheral or within the target organ itself, could evoke major alterations in biological activity.

Receptor Binding

Androgen action is initiated via binding of the compound to the androgen receptor and translocation of the steroid-receptor complex into the nucleus (*5, 18, 22, 24-26*). However, despite their similarity in one-dimensional structure, not all steroids induce androgenic responses nor do all tissues respond. This specificity is, in part, due to the presence of hormone-specific receptor proteins in target tissues. As receptor assays become more

and more sensitive, androgen receptors have been found in an increasing number of tissues. These include the male accessory sex tissues, testes, hypothalamus, preoptic region, pineal organ, pituitary, muscle, skin, uterus, vagina, kidney, and preputial, sebaceous, and submaxillary glands of a variety of species. The androgen receptor is thought to be similar in all tissues of an individual and is remarkably similar between species. In addition, most of the general aspects of hormone binding and mechanism of action are quite similar between the different classes of steroids.

When studying a new receptor in a "new" tissue it is important to differentiate steroid receptors from other proteinaceous macromolecules, such as enzymes or carrier proteins, which may also bind steroids. A steroid receptor can be defined as that soluble, cytoplasmic protein which, when combined with a specific steroid, enters the nucleus and, upon binding of the complex to specific acceptor sites on chromatin, stimulates nuclear responses and a new pattern of gene expression.

Numerous *in vitro* studies of receptors for all classes of steroids have demonstrated that there are some basic biochemical features that are common to all of them. Although it is not known if the receptor has these characteristics in the intact cell, they should be demonstrated *in vitro* before any new binder is called a receptor. These characteristics include sedimentation in the 8 S region of low-salt sucrose gradients, formation of a 4-5 S complex upon the addition of salt, an acidic PI, heat lability, high-affinity (nM) low-capacity binding, and the intranuclear transfer of the steroid-binder complex into the nucleus where it is retained with high affinity.

The androgen receptor binds both testosterone and 5α-DHT with high affinity; the K_d is in the nanomolar range. A variety of other compounds, not directly related to testosterone, have been shown to exert androgenic or antiandrogenic activities via binding to the androgen receptor. These include the synthetic progestins which may be androgenic, such as medroxyprogesterone acetate, or antiandrogenic, such as cyproterone acetate, the antiandrogen, BOMT (6α-bromo-17β-hydroxy-17α-methyl-4-oxa-5α-androstan-3-one) and the nonsteroidal compounds, flutamide and the antimineralocorticoid, spironolactone. There are also several reports suggesting that pesticides and herbicides may bind to the androgen receptor (*27-33*). Those compounds with androgenic activity act in a manner similar to testosterone. It is not known, however, why, in the case of the antiandrogens, the receptor complex is inactive. There may be several different explanations.

In a study of the binding of mouse kidney androgen receptors to a variety of progestins which have different effects on androgen action, we could not correlate relative binding affinities of the steroids, as measured in cytosol, with their biological activity, androgenic, antiandrogenic, or inactive (Table 1) (*4*). This was not surprising, considering the number of other steps at which these compounds could act to affect androgen action. Results from other investigators suggest that additional parameters may be more informative in predicting and understanding these multiple biological effects. Raynaud and colleagues have correlated antiandrogenic activity with fast dissociation rates (*34*). Sherman et al. proposed an allosteric model for determining the biological activity of the steroid receptor complex (*35*). The receptor may be either in the active or inactive form, with the biological action of the complex being determined by the percent of receptor in the active form. In other systems, antiestrogenic activity has been ascribed to lack of entry of the receptor complex into the nucleus, prolonged retention of the complex in the nucleus, binding to a different class of intranuclear acceptor sites, or a failure to stimulate regeneration of cytoplasmic receptors. The biochemical difference between a steroid receptor when it is bound to an agonist versus an antagonist has yet to be elucidated. A major hindrance to the indepth characterization of the binding of the androgen receptor with various compounds as well as nuclear acceptor site-receptor interactions is the absence of a purified androgen receptor.

Nuclear Response

The binding of androgen by the receptor is necessary for *in vivo* receptor "activation," such that now the receptor, complexed with the steroid, is translocated into the nucleus where it is bound at specific acceptor sites on chromatin (*18, 24-26, 36*). This activation step apparently represents a change in size and conformation of the receptor with the loss or gain of an additional protein moiety. The intranuclear binding of the complex results in its concentration and nuclear retention for several hours.

While the steroid is necessary for the *in vivo* activation and subsequent nuclear entry of the receptor, it is the protein and its interaction with chromatin that initiates subsequent events. The androgen insensitivity of the Tfm/Y mouse is due to the absence of androgen receptors so that target tissue nuclei remain unstimulated even in the presence of androgen. Although the activated hormone-receptor complex can bind to DNA in a

Table 1. Comparison of the relative binding affinity (RBA) for mouse kidney androgen receptors and the relative biological activity of C21 progestins.

Compound	RBA	Relative biological activity		
		Progestational	Androgenic	Antiandrogenic
Cyproterone acetate	30	Very potent	0	+ + +
Medroxyprogesterone acetate	8	2,000	+ + +	0
17α-Hydroxy-6α-methylprogesterone	12	—	0	0
6α-Methylprogesterone	36	150	+ +	+ + +
17α-Acetoxyprogesterone	14	2,500	+ +	0
17α-Hydroxyprogesterone	4	1	0	0
Progesterone	3	100	0	0

nonspecific fashion, the chromosomal, non-histone proteins play an active role in regulating receptor interaction at specific, appropriate sites within the genome of target tissues.

The processes involved in the interaction of the steroid receptor complex and the acceptor sites on chromatin are not well understood. Target organ chromatin does provide greater specificity of acceptor sites than does nontarget organ chromatin. However, the overall apparent heterogeneity in nuclear binding sites results in classes of acceptor sites which differ in their binding affinity. The biological importance of these different classes of acceptor sites is not known.

One of the earliest responses to androgen treatment that can be detected is an increase in the activity of nuclear RNA polymerase I (nucleolar) and II (nucleoplasmic) activity. We have shown that a single dose of testosterone results in a biphasic increase in RNA polymerase I and II activity in mouse kidney nuclei (Fig. 3) (5). The increase in activity can be detected as early as 15 min after hormone administration and peaks after 30-60 min and again after 12-20 hr. This response was not seen in androgen-insensitive Tfm/Y mice.

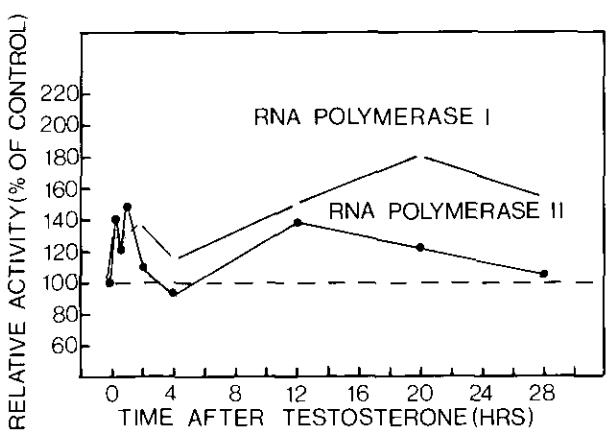


FIGURE 3. Effect of testosterone (1 mg) on RNA polymerase activities in mouse kidney nuclei (5).

Interestingly, the androgenic progestin, medroxyprogesterone acetate (MPA) and the antiandrogenic progestin, cyproterone acetate, also induced an increase in polymerase activity. The increase in polymerase activity in response to steroid treatment may be due to an activation or an increase in the number of polymerase molecules, to an increase in template capacity or to an increase in the rate of mRNA chain elongation. When testosterone and MPA were compared they were found to differ in their effects on chromatin template activity of specific polymerase molecules and chain elongation. It is probable that different hormones in different tissues exert different effects on each of these parameters. Another very early and probably very important intranuclear response to androgen treatment is the phosphorylation of nuclear non-histone proteins including the polymerases.

Although it has been known for some time that androgen treatment results in an increase in mRNA synthesis in target organs, it has not been until recently that several specific mRNA's have been identified. These include mRNA for major urinary protein (MUP) in mouse liver (37), $\alpha_{2\mu}$globulin in rat liver (38), aldolase (39) and prostatic binding protein (40) in rat prostate and β-glucuronidase (41) and a protein of unknown function (42) in mouse kidney.

Concomitant with the increase in specific mRNA's, there is a general increase in rRNA. The overall result is increased synthesis on cytoplasmic polysomes of specific proteins which are characteristic for the individual target tissues involved. In some tissues a later response is the increase in DNA polymerase and cell division.

Biological Response

Even after the steroid receptor complex has finally induced a nuclear response there are specific factors which regulate the biological response of individual end points. We have been interested in

studying some of the genetic factors regulating specific androgen effects in the mouse.

As mentioned earlier, a number of different proteins in mouse kidney respond specifically to androgen stimulation. In no instance was a response obtained in Tfm/Y mice. β-Glucuronidase has been a most useful endpoint to study because of its known genetic regulation. Alleles for the structural as well as the regulatory gene have been identified (*8*). The regulatory gene, Gur, is specific for β-glucuronidase response to androgens in the kidney and represents the only gene known to regulate steroid response. Most mouse strains have been found to be high (Gur^a) or low (Gur^b) responders. This response is regulated at the level of transcription since the degree of response is directly correlated with differences in $mRNA_{\beta\text{-glucurpnidase}}$ and β-glucuronidase synthesis. We have shown that Gur also regulates the response of glucuronidase to the androgenic progestin, medroxyprogesterone acetate (Fig. 4) (*4*). This is further evidence that Gur is related to androgen receptor-chromatin interaction.

Another as yet incompletely described, regulation of glucuronidase response is hypophysectomy (*8*). Although the androgen response of several other renal enzymes remains unaffected, the response of glucuronidase synthesis and activity is greatly reduced. Even so, the difference between Gur^a and Gur^b responses is maintained. Administration of growth hormone or prolactin will partially restore the response.

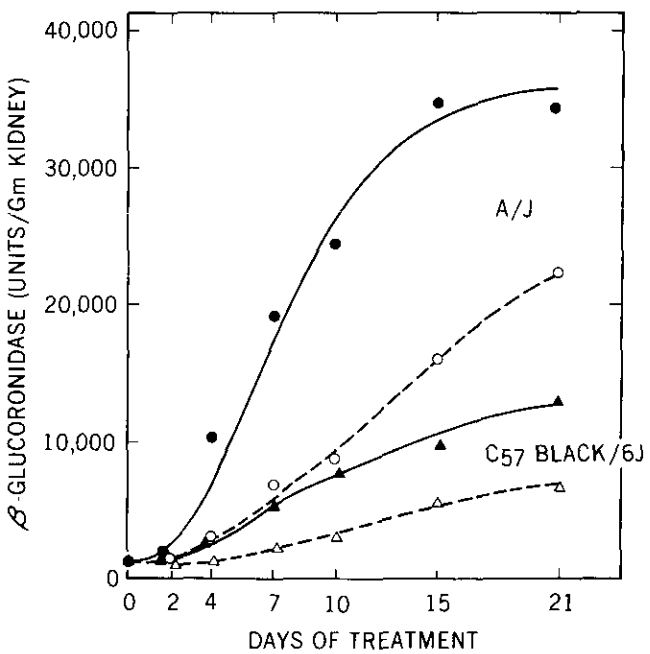


FIGURE 4. Effect of (—) testosterone and (--) medroxyprogesterone acetate on mouse kidney β-glucuronidase activity in a strain with $Gur^{a/a}$ (A/J) and $Gur^{b/b}$ (C57BL/6 J) type response. Steroid (1 and 3 mg, respectively) was administered daily (*4*).

We have also done a few studies in the area of toxicology. $CHCl_3$ and dimethylnitrosamine (DMN) are extremely active in male kidney but not so in female kidney. Both the toxicity of $CHCl_3$ and the metabolism of DMN to a mutagen can be induced in females by treatment with testosterone or androgenic progestins (*43-45*). These effects of androgens are mediated via the androgen receptor as suggested by the insensitivity of the Tfm/Y mouse and the inhibition by antiandrogens. In both instances, marked interstrain differences were noted. In neither case did the differences correlate with differences in glucuronidase responsiveness. The effects on liver were not affected by androgen treatment. Species and strain differences were also noted for liver when the hepatic response of ethylmorphine *N*-demethylase to testosterone and several synthetic C_{21} progestins was studied (*46*). It is, thus, evident that androgens can have important effects on drug metabolism or the manifestation of toxic effects.

From the above examples it should be obvious that tissue and specific genetic factors may have major effects on the expression of the final biological response. In the study of the effects of any androgen or other compound capable of affecting androgen action it is important to study the response in several tissues and, if possible, in several strains or species. The latter is particularly important when animals as unique as inbred strains of mice are used. Animals with definitive mutations may offer useful probes for the further understanding of the biochemical action of androgens and the factors that affect it.

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